Absence of reactivation or of mobilization of HSV-1 genomes following infection of the non-replicative HSV-1 vector for gene therapy. A literature review.

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Abstract Wild type Herpes Simplex Virus type 1 (HSV-1) is a very common virus. Using non-replicative vectors derived from HSV-1 for gene therapy approaches thus requires that neither reactivation of, nor recombination with preexisting infection occurs after administration of such therapy. Fortunately, many experiments from various groups have shown that such undesirable mechanisms seem very unlikely if even possible. The present document presents a summary of existing publications covering this topic.

Natural herpesvirus infections.

Humans are the natural host of herpes simplex virus types 1 and 2 (HSV-1, HSV-2). HSV-1 resides in greater than 60% of the world's population, while epidemiologic studies indicate that, among 15-49 years old, only 11.7 % of the population is infected with HSV-2. These viruses generally infect the facial (mainly HSV-1) or genital (mainly HSV-2) mucosa, where they accomplish a few cycles of virus multiplication before infecting the sensory neurons that innervate the infected area. The virus particles are then retrogradely transported to the nucleus of these neurons, which localize respectively to the trigeminal or the sacral dorsal root ganglia (DRG), where their genomes can establish life-long latent infections. From latency, both HSV serotypes can reactivate and cause peripheral pathology following anterograde trafficking from sensory neurons to the site of primary infection. Under very rare circumstances, HSV-1 can migrate to the central nervous system (CNS), via trans-synaptic spread, sometimes causing herpes simplex encephalitis (HSE), while HSV-2 is more commonly associated to meningitis (***Menendez and Carr, 2017***). There is however increasing evidence that HSV can cause asymptomatic infections of the CNS and can remain in latency in the brain (***Itzhaki, 2014***).

Potential risks associated to the use of non-replicative vectors

There are two main theoretical risks associated to the use of a non-replicative vector. Firstly, since many people harbor latent wild-type HSV in peripheral ganglia there could be a risk for an injected

non-replicative therapeutic vector to induce latent genome reactivation in ganglionic neurons that had been previously infected with wildtype HSV-1 or 2. The second concern is the possibility that the therapeutic vector genome, once established in latency following treatment, could be remobilized following primary infection with wildtype HSV, or by spontaneous reactivation of a wildtype HSV latent genome, taking place sometime after treatment. The term mobilization in this context comprises both recombination between virus genomes and complementation of the defective vector by the wildtype virus. In all cases, the associated risks are the unwished spread of the reactivated genomes or of recombinant genomes carrying the therapeutic transgene introduced by the non-replicative vector. However, as developed in the following paragraphs, a large body of experimental evidence and data coming from clinical observation strongly indicates that these risks are very low, if any.

Replication-defective HSV-1 vectors do not induce reactivation of latent wildtype HSV.

Many studies ascertain that superinfection with a non-replicative HSV-1 vector does not induce reactivation of a latent wildtype HSV-1 genome:

- In a study that used two rat latent infection models to evaluate the risk of reactivation by superinfection with a defective vector, adult rats were infected first with wild-type HSV-1 by cornea scarification or by intracerebral injection and, after the establishment of latency, a defective vector was injected intracerebrally. In the control group, the latent virus was reactivated by treatment of cadmium sulfate, showing that the latent genome was reactivatable. During experimentation, although the reactivated wild-type virus was readily detectable in the cadmium sulfate-treated animals, intracerebral infection with the defective vector failed to reactivate the latent virus in either the corneal model or the cerebral model. These results indicate that intracranial injection of a defective recombinant virus may bear little risk of reactivating latent wild-type virus harbored in the sensory ganglia or the brain (***Wang et al., 1997***).
- A similar observation was made by Fink and colleagues before and during a clinical trial to treat cancer-related pain using a non-replicative HSV-1 vector. First, this team performed a comparable study to that of Wang et al., 1997. To examine the possibility of reactivation of HSV-1 by a non-replicative vector, they first infected the cornea of mice with wildtype HSV-1 and then, 30 days later, they applied the non-replicative vector to the same cornea. Again, they were unable to detect reactivation of the latent wildtype HSV-1 from peripheral DRG (David Fink, personal communication).

  Fink and colleagues later undertook a phase-I dose-escalation study of NP2, a non-replicative HSV-1 vector expressing PENK (human pre-proenkephalin), to assess the safety of the defective HSV-1-based gene transfer platform in humans (Fink et al., 2011). While not directly addressing the question of reactivation of a latent genome, both HSV-1-seronegative and HSV seropositive patients have been enrolled in this clinical trial. There was no patient seroconverted from anti-HSV antibody negative at baseline to antibody positive at 1 or 4 months after inoculation with NP2. Among the patients who were anti-HSV antibody positive at enrollment none showed any increase in anti-HSV antibody titer after treatment with NP2. In addition, no HSV DNA was detected by PCR in the

blood or urine of subjects 1, 7, or 14 days following NP2 delivery. No treatment-related serious adverse event was reported during the 4-month follow-up period. Of the 10 patients enrolled, 8 completed the 28-day study, and 4 of them completed the 4-month follow-up. In conclusion, Fink et al have not observed any evidence for reactivation of latent HSV-1.

As they comment in their study: "The safety profile observed in this study could actually be anticipated. Oncolytic recombinant HSV-1 viruses intended to kill malignant cells by limited replication had been injected directly into brain, liver, and skin tumors in more than 200 patients, without any reported test agent-related serious adverse events. Replication-competent HSV recombinants have also been tested in clinical trials as potential vaccines against genital herpes. Although these approaches have in some cases generated quite high anti-HSV antibody levels, no HSV recombinants-related serious adverse events have been reported".

- Still a further example is provided by a study conducted by Sundaresan et al, 2000, who investigated the risk of inducing reactivation of latent HSV-1 in the brain following intracerebral inoculation of an attenuated, replication-competent oncolytic HSV-1 vector. As a model for latent HSV in the brain, this team used survivors of an intracerebral inoculation of wildtype HSV-1 at the 50% lethal dose. Inoculation of a high dose of the attenuated vector at the same stereotactic coordinates did not result in reactivation of detectable infectious wildtype HSV-1 or symptoms of disease, again demonstrating that the vector was unable to induce reactivation of latent HSV-1.
- The last line of evidence supporting the low risk of reactivation of latent genomes by superinfecting HSV-1 comes from clinical observations using oncolytic viruses, which are conditionally replicative viruses able to multiply and produce progeny particles only in cancer cells. Currently, more than 50 different types of oncolytic HSV-1 have been constructed and studied in animal models of cancer. Nine of them are currently being tested in phase-I or phase-II clinical trials. Talimogene laherparepvec (T-Vec), also known as OncoVEX-GMCSF or Imlygic, has been approved in 2015 in both the US and Europe. Accordingly, several hundred cancer patients have been treated with these viruses. It must be emphasized that these oncolytic viruses are replication-competent and can infect non-cancer cells. While they cannot replicate in these cells, they can penetrate them and express at least some of their genes or transgenes. However, as far as we know, no case of reactivation of latent HSV-1 genome, nor any serious adverse event, has been reported in any clinical trials with these vectors (***Sanchala et al., 2017***).

The therapeutic vector genome should not be remobilized following primary infection or spontaneous reactivation of a wildtype HSV-1 genome.

The above-described observations constitute strong evidence in favor of the absence of reactivation of a latent wildtype genome following superinfection with a non-replicative vector, or even with an attenuated replicative vector. On the other hand, once established into latency, the therapeutic vector genome cannot spontaneously reactivate because it is non-replicative, lacking genes essential for reactivation or multiplication. But it is pertinent to inquire whether there is any risk of remobilization associated with spontaneous reactivation of wildtype genomes or with primary infection with HSV-1 taking place sometime after treatment, two situations that could result in the presence of replicative wildtype particles

within the ganglia. This question is pertinent because it is well known that different strains of HSV-1, or even different serotypes (HSV-1 x HSV-2) can recombine or can complement one another (***Casto et al., 2020***). However, recombination or complementation can take place only if the viruses coreplicate within the same cells, thus enabling physical interactions between both genomes. Again, we postulate that neither of those events represent a risk for a gene therapy strategy, and the main reason is that viruses infecting different tissues should not meet in the same neurons, thus precluding any physical interaction between the therapeutic vector and wildtype HSV-1 genomes. Sacral DRG contains sensory neurons innervating different anatomic sites. Some of these neurons innervate the bladder, others the gut, others the genital region, others the skin, and so on. Even if the bodies of all these neurons can cohabit in the same ganglia, there seem to be no connections between them. This implies that HSV particles having reached a sacral DRG via sensory neurons innervating the bladder are not normally expected to spread to neighboring sensory neurons innervating the genital region, even if they coexist in the same DRG. Even following primary infection or spontaneous reactivation of a latent wildtype genome, which can occur in neurons innervating the genital region, there is low probability that the newly formed HSV particles spread to neighboring bladder neurons within the same ganglia. Actually, most of these progeny particles will spread to neurons or tissues connected by synaptic junctions and will most probably reinfect the primary site of peripheral infection, thus producing a classical herpetic recurrence. In this context, it is worth noting to recall that in case of primary infection or reactivation of a latent wildtype HSV-1 there are several well-known specific antivirals (acyclovir, valacyclovir, famciclovir or forscarnet) that very efficiently control peripheral (or even central) herpetic infections. Indirect evidence to support the above statements comes from tracing experiments, conducted in rodent brains using replicative but attenuated HSV-1 or PRV, a virus closely related to HSV-1, either to study neuron connectivity by following virus spread (***Ekstrand et al., 2008***) or to investigate virus spread within the brain for clinical or therapeutic reasons (***Lilley et al., 2001***). In both cases, results clearly suggest that following intracranial inoculation, virus particles do not infect neighboring neurons but are transmitted essentially via synapses to post-synaptic neurons and are detected far from the site of infection. In addition, in studies envisioned to study neural circuitry in the rat CNS by coinfecting two recombinant strains of PRV, it was shown that although some neurons can be coinfected by the two strains, single infections largely predominate. Moreover, prior infection with one virus reduces the ability of neurons to replicate the other recombinant strain (***Kim et al., 1999***). It could be argued that in this study the synchronicity of infections can explain these observations in terms of interference. However, other studies suggest that latent viruses have evolved ways to evade superinfection by a non-latent virus of the same species to avoid destruction of their own host cell (***Berngruber et al., 2010***). Superinfection inhibition is common and has been shown in viruses of bacteria, plants, and animals, including HSV-1, where expression of the latency-associated (LAT) gene was shown to inhibit superinfection by another HSV-1 (***Mador et al., 2002***). Taken together, these studies indicate that the probability of the non-replicative vector to meet a replicating wildtype HSV-1 within a DRG is very low, thus precluding any type of physical interaction between both genomes, including recombination and complementation.

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